

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23407 A1

(51) International Patent Classification⁷: C07J 71/00,
9/00, A61K 31/575, 31/58, A61P 2/04, 25/28, 25/14

(21) International Application Number: PCT/GB00/03745

(22) International Filing Date:
29 September 2000 (29.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9923077.3 29 September 1999 (29.09.1999) GB

(71) Applicant (for all designated States except US): PHY-
TOPHARM PLC [GB/GB]; Corpus Christi House, 9 West
Street, Godmanchester, Cambridgeshire PE18 8HG (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BARRACLOUGH,
Paul [GB/GB]; 27 Sevington Park, Loose, Maidstone,
Kent ME15 9SB (GB). HANSON, Jim [GB/GB]; 13
Ingram Road, Steyning, West Sussex BN44 3PF (GB).
GUNNING, Phil [GB/GB]; 16 Sladwell Close, Grantch-
ester, Cambs. CB3 9NP (GB). REES, Daryl [GB/GB]; 4

Hazels Hall, Everton Road, Sandy SG19 2DD (GB). XIA,
Zongqin [CN/CN]; Flat 403, Block 8, 1 Jian-De Road,
Shanghai 200025 (CN). HU, Yaer [CN/CN]; Flat 1209,
Block 1, Lane 357, Ma-Dang Road, Sanghai 200025 (CN).
TOBIN, Andrew [GB/GB]; 16 Meadvale Road, Leicester
LE2 3WJ (GB).

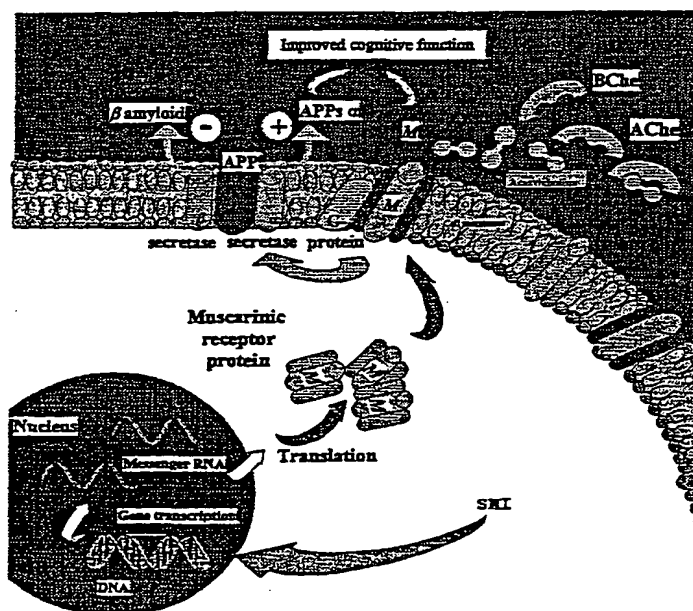
(74) Agent: ABRAMS, Michael, John; Haseltine Lake & Co,
Imperial House, 15-19 Kingsway, London WC2B 6UD
(GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: SAPOGENIN DERIVATIVES AND THEIR USE IN THE TREATMENT OF COGNITIVE DYSFUNCTION



(57) Abstract: The invention discloses the use of sapogenin derivatives in the treatment of cognitive dysfunction and similar conditions. Methods of treatment and pharmaceutical composition are also disclosed.

BEST AVAILABLE COPY

WO 01/23407 A1



Published:

- *With international search report.*
- *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SAPOGENIN DERIVATIVES AND THEIR USE IN THE TREATMENT OF COGNITIVE DYSFUNCTION

5 The present invention relates to sapogenin derivatives and their use in
treating cognitive dysfunction and allied conditions; and to compositions for use in
such treatments. The invention is also concerned with the treatment of conditions
that are characterised by a deficiency in the number or function of membrane-
bound receptors. In the following, the present invention will be described
principally with reference to the treatment of Alzheimer's disease (AD) and senile
10 dementia of the Alzheimer's type (SDAT), where deficiencies in a number of
receptor types have been demonstrated. However, it is to be understood that the
present invention relates generally to the treatment of conditions attributable to
intrinsic pathological conditions and/or exposure to adverse environmental
conditions these conditions being characterised by a deficiency in the number or
15 function of membrane-bound receptors or a deficiency in transmission at the
junctions between neurones or at the junctions of neurones and effector cells.

Conditions of the type mentioned above include Parkinson's disease, Lewi
body dementia, postural hypotension, autism, chronic fatigue syndrome,
20 Myasthenia Gravis, Lambert Eaton disease, diseases and problems associated with
Gulf War Syndrome, occupational exposure to organophosphorus compounds and
problems associated with ageing.

Alzheimer's disease (AD) and senile dementia of the Alzheimer's type
25 (SDAT) are grave and growing problems in all societies where, because of an
increase in life expectancy and control of adventitious disease, the demographic
profile is increasingly extending towards a more aged population. Agents which
can treat, or help in the management of, AD/SDAT are urgently required.

30 Age-associated memory impairment (AAMI) is a characteristic of older
patients who, while being psychologically and physically normal, complain of
memory loss. It is a poorly defined syndrome, but agents which are effective in
treatment of AD/SDAT may also be of value in these patients.

35 Research into AD/SDAT is being carried out by traditional and
conventional medical research methods and disciplines. In conventional medicine,

- 2 -

there are several approaches to the treatment of AD/SDAT. It is known that the biochemical processes subserving memory in the cerebral cortex are (at least in part) cholinergically-mediated. Those skilled in the art will know that "cholinergically mediated" mechanisms may be directly attributable to acetylcholine acting on receptors, and these are direct effects. Other, clinically useful effects may also be caused by modulation of release of acetylcholine from pre-synaptic nerve endings or inhibition of enzymes that destroy acetylcholine. These modulating factors may be exerted through neurones where the mediator is non-cholinergic; these are referred to as indirect effects. Some attempts at treatment have focussed on the role of other mediators such as 5-hydroxytryptamine, which is a mediator in other areas of brain, such as the mid-brain nuclei. However, since fibres from these areas are projected forward into the cerebral cortex where the primary transmitter is acetylcholine, attention has focussed on the management of this mediator in the search for appropriate therapeutic agents.

Cholinergic strategies for the treatment of AD/SDAT have been directed at several points along the pathway of formation, synaptic release and removal of released acetylcholine.

One approach involves treatment with high doses of lecithin and other precursors of acetylcholine. This is of limited use in producing sustained improvements in cognitive performance.

Another approach involves the use of vegetable drugs such as Polygalae root extract, which has been shown to enhance choline-acetylcholine transferase (CAT) activity and nerve growth factor (NGF) secretion in brain. Oral administration of NGF has no effect on central nervous system neurons because it is a high molecular weight protein that cannot pass through the blood-brain barrier. However, agents which can pass through the blood-brain barrier and have a stimulating effect on NGF synthesis in the central nervous system have been proposed for the improvement of memory-related behaviour.

The results of a third clinical approach, which uses cholinesterase inhibitors such as tacrine hydrochloride, have been marginally more positive than the above. Substances obtained from plants used in Chinese and Western medicine, for

- 3 -

example huperzine, galanthamine, and physostigmine have all been shown to be of some - although limited - benefit in the treatment of AD/SDAT in clinical studies and also in laboratory models. All of these substances are inhibitors of acetylcholine esterase (AChE). In patients with AD/SDAT, there may be reduced synthesis of acetylcholine (ACh), reduced efficiency in release of ACh from presynaptic stores, and a decrease in the number or function of postsynaptic (M_1) receptors. Reductions in pre-synaptic M_2 receptors have also been shown. The beneficial effect of AChE inhibitors is attributed to enhancement of acetylcholine levels at synapses in brain by slowing down the destruction of released transmitter.

Compositions which modulate cholinergic function are known to affect memory and recall. For example, nicotine stimulates nicotinic acetylcholine receptors, and the short lived memory enhancing effects of cigarette smoking are thought to be due to the effect of nicotine. Scopolamine, an antagonist of acetylcholine, will produce amnesia and impaired cognitive function manifesting in psychomotor tests as a prolongation of simple reaction times, possibly as a result of impaired attention, and is used for this purpose as an adjunctive analgesic treatment. The amnesic effect of scopolamine can be antagonised by nicotine.

There are two families of nicotinic receptor subtypes (α and β), and each includes four subgroups which differ in ligand specificity. The role of nicotinic receptors in the CNS is not well understood at the molecular level. It is possible that agents binding to nicotinic receptors may modify the rate of turnover at muscarinic receptor sites in brain. Nicotinic receptors are ligand-gated ion channels, and their activation causes a rapid (millisecond) increase in cellular permeability to Na^+ and Ca^{++} , depolarisation and excitation.

Another class of cholinergic receptors can be stimulated by muscarine. Such muscarinic (M) receptors are G protein-coupled receptors. Responses of muscarinic receptors are slower; they may be excitatory or inhibitory. They are not necessarily linked to changes in ion permeability. Five types of muscarinic receptors have been detected by cholinergic receptor cloning, and are designated as m_1 - m_5 . Pharmacological effects are associated with four of the cloned receptors and they are designated as M_1 - M_4 based on pharmacological specificity.

Using specific receptor proteins and monoclonal antibodies, it has been

- 4 -

possible to further localise muscarinic receptors in brain as m_1 (postsynaptic) and m_2 (presynaptic). In heart, M_2 receptors are postsynaptic. Presynaptic muscarinic receptors are thought to be inhibitory, the binding of ACh to these receptors attenuating the release of further ACh to provide a negative feedback mechanism for ACh release. Selective M_2 receptor antagonists which are preferentially distributed to the brain may therefore be useful in treating Alzheimer's disease.

It is known that, in disease states such as AD/SDAT, there is general neuronal loss and deficits in cholinergic nerve function. It has been speculated that the high affinity nicotinic binding sites in the remaining cholinergic neurons might be converted to low affinity binding sites in treating such diseases, thereby sustaining transmitter release. By lowering the affinity of the nicotinic binding sites, a quick desensitising process is avoided.

Agonist activation at nicotinic receptors in brain has rapid onset and offset. A decreased affinity of the nicotinic receptors will reduce the desensitisation process. Schwarz R.D. et al (J. Neuro Chem 42, (1984), 1495-8) have shown that nicotine binding sites are presynaptically located on cholinergic (and also 5-hydroxytryptaminergic and catecholaminergic) axon terminals. A change in high affinity binding sites on AD/SDAT may also induce a change in the modulatory effect the nicotinic binding sites may have on other transmitter systems.

Presynaptic cholinergic mechanisms are also under inhibitory control by GABAergic neurons and this inhibition is thought to be intensified in AD/SDAT. Removal or reduction of this inhibition intensifies presynaptic cortical cholinergic activity and enhances cognitive processing.

The interactions of interneuronal fibres innervated by nicotine (reducing binding affinity), and dis-inhibition of GABAergic fibres both have a presynaptic locus.

This is a simplistic model of central transmission, but provides a framework for understanding the attempts which have been made to increase the effective concentration of acetylcholine in central synapses. This further illustrates the concept of direct and indirect action. There are disadvantages attaching to the three conventional therapeutic approaches to AD/SDAT treatment mentioned above:

- 5 -

ACh precursor supplementation, agonist replacement and acetylcholine esterase inhibition. These treatments may result in a short-term increase in the availability of ACh which may activate feedback mechanisms resulting in the desensitisation of postsynaptic receptors. On theoretical grounds, long term benefits would not be predicted and when treatment is interrupted, any benefits in management of AD/SDAT and AAMI disappear and the condition may even be aggravated.

It has been shown that a compound with M_1 agonist and M_2/M_3 antagonist activity improved cognitive performance in SDAT patients (Sramak et al, Life Sciences vol. 2, No. 3, 195-202, 1997). However, this compound causes unacceptable cholinergic side effects, such as fatigue, diarrhoea and nausea.

A more radical approach to AD/SDAT and AAMI aims to increase the number of postsynaptic (M_1) receptors, in brain. It is known from Chinese Patent No. CN1096031A, that sarsasapogenin (SaG) can up-regulate M_1 cholinergic receptors.

Patent applications have been published which claim the usefulness of a number of steroid sapogenins having spirostane, furo-spirostane, spirosolane or solanidine structures in the treatment of diseases including SDAT. Two patent publications are of particular relevance here: Chinese patent publication No CN1096031A claims the use of the spirostane sapogenin, sarsasapogenin, in the treatment of SDAT. The disclosure in this document, however, is brief. The other document of relevance is patent publication DE 4303214A1 which claims the use of a very wide range of saponins and sapogenins in the treatment of a whole range of diseases that the inventors consider to be of viral origin. This disclosure is however of dubious value in that it is well recognised that there is no infective element to a very large number of the conditions that are characterised by deficient synaptic transmission and thus the basic premise of the alleged invention is flawed. In addition they present no data of any kind that allows one skilled in the art to be able select a preferred compound from the large number that are claimed.

The inventors have found that certain sapogenin derivatives exhibit the ability to regulate receptors. In particular, these compounds have been found to increase the number of M_2 receptors in the brain. Thus, according to one aspect of the invention, there is provided the use of a sapogenin derivative of general

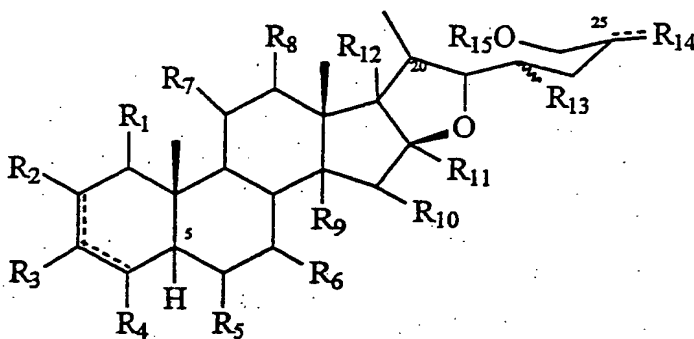
formula (I) in the manufacture of a medicament for the treatment of a condition characterised by a deficiency in membrane-bound receptor number or function.

Those skilled in the art will be aware of the relationship between saponins and their sapogenins, and that the latter tend to be fat-soluble whereas the saponins tend to be water-soluble. Sapogenins are therefore better able to cross the blood-brain barrier. The skilled man will also be aware of the epimerisation of certain sapogenins under conditions of acid hydrolysis.

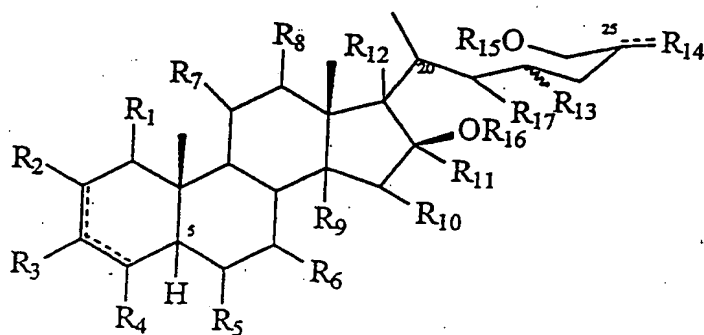
The variation in pharmacological properties and pharmacodynamic actions of various types of sapogenins underlines the need for selection of those agents which are most useful in the treatment of AD/SDAT. The discovery of novel facts about the action of sapogenin derivatives has made it possible to determine which substances are most useful for the treatment of AD/SDAT and the like.

The inventors have found that the above-described properties are exhibited by sapogenin derivatives wherein the E and/or F ring of the fused ring system has been cleaved.

Accordingly, the sapogenin derivatives of interest in this invention have the following general formulas (I) or (II):



I



II

5

10 their stereoisomers and racemic mixtures, their pharmaceutically acceptable pro-drugs and salts.

In the general Formula (I):

15 -R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, are, independently of each other, either H, OH, =O, and OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;

-R₉, R₁₂, R₁₁, R₁₃ can be either a H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;

-R₁₄ = optionally substituted alkyl group

20 -R₁₅ = H, optionally substituted alkyl, optionally substituted acyl;

-the hydrogen at C5 may be either α or β
and represents an optional double bond,

25 Preferably, in the general formula (I):

-R₄, R₉, R₁₂, R₁₃ = H

-R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₁₀, can be independently of each other either H, OH, =O, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;

30 -R₁₁ = H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl group;

-R₁₄ = optionally substituted alkyl;

-R₁₅ = H, optionally substituted alkyl or optionally substituted acyl,

-the hydrogen at C5 may be either α or β

35 and represents an optional double bond,

- 8 -

In the general formula (II):

- R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₇, are, independently of each other, either H, -OH, =O, or OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
- R₉, R₁₂, R₁₁, R₁₃ can be either a H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
- R₁₄ = optionally substituted alkyl group;
- R₁₅ = H, optionally substituted alkyl or optionally substituted acyl;
- R₁₆ = H, optionally substituted alkyl or optionally substituted acyl;
- the hydrogen at C5 may be either α or β
- and represents an optional double bond,

Preferably, in the general formula (II):

- R₄, R₉, R₁₂, R₁₃ = H
- R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₁₀, R₁₇, can be independently of each other either H, OH, =O, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
- R₁₁ = H, OH, OR where R = alkyl or acyl group;
- R₁₄ = optionally substituted alkyl;
- R₁₅ = H, optionally substituted alkyl or optionally substituted acyl;
- R₁₆ = H, optionally substituted alkyl or optionally substituted acyl;
- the hydrogen at C5 may be either α or β
- and represents an optional double bond;

More preferably, in the general formula (II):

- R₁ = R₂ = R₄ = R₅ = R₆ = R₇ = R₈ = R₁₀ = R₁₁ = R₉ = R₁₂ = R₁₃ = R₁₅ = R₁₆ = R₁₇ = H,
- R₃ = H, OH, or OCOCH₃, or =O
- R₁₄ = CH₃

As used hereabove and hereafter:

35

"Acyl" means an H-CO- or Alkyl-CO- group wherein the alkyl group is as herein

described. Preferred acyls contain a lower alkyl. Exemplary acyl groups include formyl, acetyl, propanoyl, 2-methylpropanoyl, butanoyl and palmitoyl.

5 "Alkyl" means an aliphatic hydrocarbon group which may be straight or branched having about 1 to about 20 carbon atoms in the chain. Preferred alkyl groups have 1 to about 12 carbon atoms in the chain. Branched means that one or more lower alkyl groups such as methyl, ethyl or propyl are attached to a linear alkyl chain. "Lower alkyl" means about 1 to about 4 carbon atoms in the chain which may be straight or branched. Exemplary alkyl groups include methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, 10 *t*-butyl, *n*-pentyl, 3-pentyl.

"Optionally substituted" means that the said group may be substituted with one or more substituents which may be the same or different, and include halo, alkyl, cycloalkyl, hydroxy, alkoxy, amino, acylamino, aryl, aroylamino, carboxy, 15 alkoxycarbonyl, aralkoxycarbonyl, heteroaralkoxycarbonyl, optionally substituted carbamoyl.

The term "pharmaceutical composition" means a composition comprising a compound of formula I or II and at least one component selected from the group comprising 20 pharmaceutically acceptable carriers, diluents, adjuvants, excipients, or vehicles, such as preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms.

25 "Pharmaceutically acceptable" means it is, within the scope of sound medical judgement, suitable for use in contact with the cells of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio.

30 "Pharmaceutically acceptable dosage forms" means dosage forms of the compound of the invention, and includes, for example, tablets, dragees, powders, elixirs, syrups, liquid preparations, including suspensions, sprays, inhalants tablets, lozenges, emulsions, solutions, granules, capsules and suppositories, as well as liquid 35 preparations for injections, including liposome preparations. Techniques and formulations generally may be found in Remington, Pharmaceutical Sciences, Mack

Publishing Co., Easton, PA, latest edition.

5 "Pharmaceutically acceptable prodrugs" as used herein means those prodrugs of the compounds useful according to the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" means compounds that are rapidly transformed *in vivo* to yield the parent compound of the above formula, for example by hydrolysis in blood. Functional groups which may be rapidly transformed, by metabolic cleavage, *in vivo* form a class of groups reactive with the carboxyl group of the compounds of this invention. Because of the ease with which the metabolically cleavable groups of the compounds useful according to this invention are cleaved *in vivo*, the compounds bearing such groups act as pro-drugs. A thorough discussion of prodrugs is provided in the following: Design of Prodrugs, H. Bundgaard, ed., Elsevier, 1985; Methods in Enzymology, K. Widder et al, Ed., Academic Press, 42, p.309-396, 1985; A Textbook of Drug Design and Development, Krogsgaard-Larsen and H. Bundgaard, ed., Chapter 5; Design and Applications of Prodrugs p.113-191, 1991; Advanced Drug Delivery Reviews, H. Bundgaard, 8, p.1-38, 1992; Journal of Pharmaceutical Sciences, 77, p. 285, 1988; Chem. Pharm. Bull., N. Nakaya et al, 32, p. 692, 1984; Pro-drugs as Novel Delivery Systems, T. Higuchi and V. Stella, Vol. 14 of the A.C.S. Symposium Series, and Bioreversible Carriers in Drug Design, Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press, 1987, which are incorporated herein by reference.

30 "Pharmaceutically acceptable salts" means the relatively non-toxic, inorganic and organic acid addition salts, and base addition salts, of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds. In particular, acid addition salts can be prepared by separately reacting the purified compound in its free base form with a suitable organic or inorganic acid and isolating the salt thus formed. See, for example S. M. Berge, et al., Pharmaceutical Salts, J. Pharm. Sci., 66: p.1-19 (1977) which is incorporated herein by reference. Base addition salts can also be prepared by separately reacting the purified compound in its acid form with a suitable organic or inorganic base and isolating the salt thus formed. Base addition salts include pharmaceutically acceptable

- 11 -

metal and amine salts.

Sapogenin derivatives of interest in the present invention may occur naturally in a range of plant species, notably from the genera Smilax, Asparagus, Anemarrhena, Yucca and Agave. The species presently of greatest interest include Smilax regelii
5 Kilip & Morton - commonly known as Honduran sarsaparilla; Smilax aristolochiaefolia Miller - commonly known as Mexican sarsaparilla; Smilax ornata Hooker - commonly known as Jamaican sarsaparilla; Smilax aspera - commonly known as Spanish sarsaparilla; Smilax glabra Roxburgh; Smilax febrifuga - Kunth -
10 commonly known as Ecuadorian or Peruvian sarsaparilla; Anemarrhena asphodeloides Bunge; Yucca schidigera Roezl ex Ortgies; and Yucca brevifolia Engelm.

Sapogenin derivatives which may be of interest may also occur naturally in other genera, for example Dioscorea, Trillium, Solanum, Strophanthus, Digitalis and Trigonella. However, some sapogenin derivatives from these sources possess
15 undesirable properties and are thus not recommended for use in the invention.

Sapogenin derivatives of the invention may also be commercially available; suppliers are well-known from the one skilled in the art and may include Sigma Aldrich, Research Plus Inc., Steraloids Inc., etc...

20 According to a further aspect of the invention, there is provided a process of preparation of the compounds of the invention.

Substituted sapogenins of the present invention may be prepared by synthetic methods. For instance, they may be prepared from unsubstituted sapogenin
25 derivatives, which may occur naturally or be commercially available, as stated above.

Starting from these unsubstituted sapogenins, the reaction may involve at least one substitution step, wherein the functional group is substituted on the sapogenin derivative; usually, the starting product is an unsubstituted sapogenin having the required stereochemistry, and the reaction may involve the substitution of one OH-
30 group by the functional radical desired; smilagenin and epismilagenin are preferred as starting products.

Compounds useful according to the invention may be prepared by the application or adaptation of known methods, by which is meant methods used heretofore or described in the literature, for example those described by R. C. Larock
35 in Comprehensive Organic Transformations, VCH publishers, 1989.

In the reactions described hereinafter it may be necessary to protect reactive

functional groups, for example hydroxy or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions. Conventional protecting groups may be used in accordance with standard practice, for examples see T.W. Green and P.G.M. Wuts in "Protective Groups in Organic Chemistry" John Wiley and Sons, 1991; J. F. W. McOmie in "Protective Groups in Organic Chemistry" Plenum Press, 1973.

The compound thus prepared may be recovered from the reaction mixture by conventional means. For example, the compounds may be recovered by distilling off the solvent from the reaction mixture or, if necessary after distilling off the solvent from the reaction mixture, pouring the residue into water followed by extraction with a water-immiscible organic solvent and distilling off the solvent from the extract. Additionally, the product can, if desired, be further purified by various well techniques, such as recrystallization, reprecipitation or the various chromatography techniques, notably column chromatography or preparative thin layer chromatography.

According to a further aspect of the present invention, there is provided a pharmaceutical composition having cognitive function enhancing properties which comprises an effective amount of a sapogenin derivative of the invention.

In a still further aspect, the sapogenin derivatives of the present invention are steroidal; they are preferably non-oestrogenic in effect.

In another aspect, the invention provides a pharmaceutical composition having cognitive function enhancing properties which comprises an effective amount of a sapogenin derivative of the invention in the form of an extract derived from a plant of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave.

It will be appreciated that the invention embraces within its scope the use of the compositions defined above. Thus, according to a fifth aspect, the present invention provides a method of enhancing cognitive function which comprises administering to a human or animal an effective dosage of a composition of the invention.

The invention also provides a method of enhancing cognitive function in a human or non-human animal, which comprises administering an effective dose of

- 13 -

sapogenin derivatives of the invention. Also, it concerns the use of the sapogenin derivatives of the invention in food product or beverage for enhancing cognitive function.

5

As used herein, the term "cognitive function" refers to functions such as thinking, reasoning, remembering, imagining and learning.

10

According to a further aspect, the invention also relates to composition having cognitive function enhancing properties which comprises at least two, preferably two, sapogenin derivatives of the invention.

15

20

In identifying compounds that would have use in the treatment of SDAT and other diseases characterised by reductions in receptor numbers or synaptic transmission, the inventors have given consideration to the need to identify compounds that would have the desired effect but would be devoid of any oestrogenic effects, as these would be unacceptable, particularly in male patients. A number of the compounds claimed to have activity in patent application DE 4303214A1 have marked oestrogenic activity and are therefore unacceptable. Preferably, sapogenin derivatives of the present invention however, does not display oestrogenic activity. In addition these compound were tested at other steroid receptors and were found to have no activity at any of the following receptors:

25

Progesterone
Glucocorticoid
Testosterone

30

Sapogenin derivatives of the present invention have also been tested for activity in a number of in-vitro assays. The assays/experiments that were considered of key importance in determining possible activity in the elevation of membrane bound receptor numbers were as follows:

35

Chinese hamster ovary (CHO) cells transfected with the a DNA fragment coding for a muscarinic receptor. The cell line used for the majority of the experiments was a cell line expressing the m2 receptor.

- 14 -

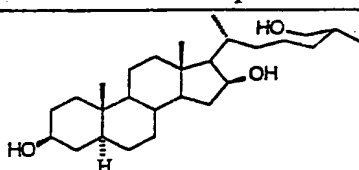
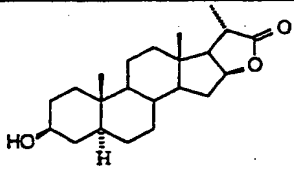
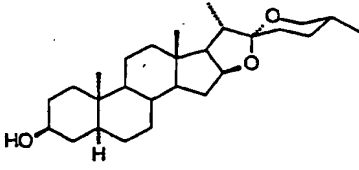
The methods and the results of these experiments are now described in turn.

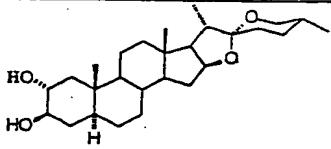
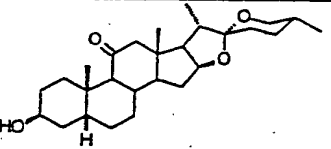
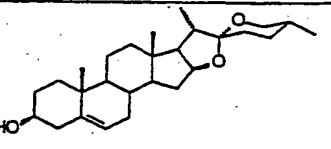
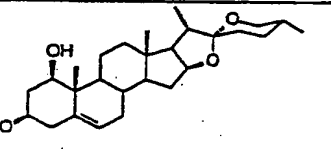
CHO cell line experiments

The effects of various compounds on the expression of m2 receptors on CHO cells transfected with DNA for the m2 receptor were investigated. Receptor numbers were assayed using tritiated QNB binding and subtracting non-specific binding. Compounds were dissolved in DMSO and DMSO was used as a control. Compounds were tested at a range of final concentrations. Compounds were also tested in the presence and absence of tamoxifen to try to distinguish an oestrogen receptor mediated mechanism.

The results are summarised in the Table 1 below. Compounds are active when the effect on receptor expression given as a percentage increase compared to control is more than 15%.

Table 1 Effects of sapogenin derivatives on the expression of m₂ receptors on CHO cells

Compound	Molar concentration	Activity
 E/F-Seco-tigogenin	10^{-5}	Active
 F-Nor-tigogenin	10^{-5}	Not active
 Tigogeuin	10^{-5}	Not active
	10^{-6}	Not active

5	 Gitogenin	10^{-5}	Not active
10	 11-Ketotigogenin	10^{-5}	Not active
15	 Diosgenin	10^{-5}	Not active
		10^{-6}	Not active
20	 Ruscogenin	10^{-5}	Not active
		10^{-6}	Not active

25

30 Thus the experiments indicate that the sapogenin derivatives of the invention were able to increase the number of muscarinic receptors expressed on the surface of CHO cells cultured in-vitro. The effect was not antagonised by tamoxifen, indicating that the mechanism involved did not involve the oestrogen receptor.

35 It appears from the experimental work conducted that the compounds of this invention act to normalise muscarinic receptor number - i.e. they tend to prevent decline in

-16-

receptor number with time, and also tend to restore receptor number to normal levels when given to cells in which the receptor number is depressed.

5 It is speculated here that the effect of the active compound claimed in this patent may operate through an effect on G protein and that the effects on receptor numbers are secondary to an effect on G-protein. When a membrane bound G-protein linked receptor is stimulated two basic sets of events are initiated: the effector response; and the internalisation of the receptor. The subsequent processing of the receptor to the state where it is again in a form on the cell surface or other membrane surface where
10 it can interact with another receptor ligand appears to be subject to a number of factors. A number of these factors or mechanisms appear to be G-protein linked. There is evidence that activation of m_3 receptors may have an effect on G-protein expression or levels. It is speculated that the actions of the compounds described in this patent may due to an interaction in the processes of receptor regeneration, G-protein linkage
15 or G-protein homeostasis.

An alternative hypothesis is that the compounds are increasing the synthesis or release or a decreased rate of degradation of neurotropic factors such as brain derived growth factor and/or nerve growth factor. These effects on growth factors
20 might be due to an effect of the compound on a cytosolic or nuclear receptor or the binding of a compound to a promoter region with a consequent effect directly on the rate of production of mRNA for the growth factor or as a consequence of increasing the production of another material factor such as G-protein or finally the effects may be secondary to an effect on receptor or G-protein procession.

25 The increased expression and/or abnormal processing of the amyloid precursor protein (APP) is associated with the formation of amyloid plaques and cerebrovascular amyloid deposits which are the major morphological hallmarks of Alzheimer's disease. Of particular interest are the processes regulating the proteolytic cleavage of APP into amyloidogenic and nonamyloidogenic fragments. The cleavage of APP by
30 the enzyme α -secretase within the β -amyloid sequence of the protein results in the formation of a non amyloidogenic C-Terminal fragment, and the soluble APP α fragment; this latter fragment has been shown to have neurotropic and neuroprotective activity as well as to enhance memory in mice when injected intra-cerebro-ventrically (ICV). In contrast, processing of APP by β -secretase exposes the N-terminus of β -
35 amyloid which is released by γ -secretase cleavage at the variable C-terminus. The

- 17 -

resulting β -amyloid peptides, which contain 39-43 amino acids, have been shown to be neurotoxic and to accumulate in plaques which interfere with inter-neurone connections.

5 A number of studies have shown that stimulation of the protein-kinase (PKC) linked muscarinic M_1 and M_2 receptors results in an increase in α -secretase activity. As a consequence processing of APP to APPs α with its neuroprotective effects is increased. In parallel, processing of APP by β - and γ -secretase is decreased and there is a consequential reduction of β -amyloid. Other transmitters such as nerve growth
10 factor (NGF) and brain derived neurotropic factor (BDNF) as well as bradykinin and vasopressin may have similar effects in increasing the proportion of APP processed to APPs α . There may be a number of factors involved in the effects of NGF which may include binding of the factor to the tyrosine kinase receptor (TrkA) and the stimulation of phospholipase C γ with subsequent phosphorylation and activation of
15 protein kinase C (PKC) and increase in relative activity of α -secretase.

Any treatment which increases activity of protein-kinase C selectively in brain might therefore be expected to be of use in the management of Alzheimer's disease. Until recently agonists selective at the M_1 receptor have not been available. Non-
20 selective agonists would be expected to stimulate pre-synaptic M_2 receptors which cause negative feedback and hence would further severely impair muscarinic transmission. Selective agonists at the M_1 receptor are now becoming available (talsaclidine) and such agents are under investigation for the treatment of AD. There is however, a substantial risk that, as with the chronic administration of any receptor
25 agonist, the clinical benefits seen will be severely limited in terms of the size of benefit by reducing receptor numbers or reducing sensitivity and in terms of side effects due to lack of receptor specificity. Thus compounds as described in this invention, which selectively regulate muscarinic receptor numbers or function would be expected to be devoid of the problems seen with a muscarinic agonist and hence
30 have particular utility. Indeed the benefits may be seen in three parts as follows.

1. A selective increase in M_1 receptor numbers leading to increased synaptic transmission. Chronic administration of a selective agonist will, at best, have no adverse effect on transmission;

2. Secondary to the increased receptor numbers, an increase stimulation of PKC with

-18-

a consequential increase in α -secretase activity, leading to:

2.1 A reduced production of β -amyloid and a consequent reduction of plaque formation and neuronal loss;

2.2 An increase in APPs α and a consequent improvement in cerebral function as witnessed by an improvement in short and long term memory.

In order to illustrate the invention further by way of non-limiting example, reference will now be made to the accompanying drawings and to the Example which follows; in the drawings:

FIGURE 1 illustrates the results obtained in Example 1 below;

FIGURE 2 illustrates a hypothetical mode of action for sapogenin derivatives;

Referring to Fig.2, a diagrammatic representation of the function of sapogenin derivatives of the invention is shown. It is believed that sapogenin derivatives act primarily on cell nuclei; the invention is not, however, limited to any particular mode of action. The observed increase in muscarinic receptor number consequential upon administration of sapogenin derivatives is interpreted as leading to increased expression of muscarinic receptor protein. The possible link between the secretases and β -amyloid protein formation (discussed above) is indicated in the drawing.

The following Example is provided to illustrate the invention in a non-limiting manner.

Example 1

In a CHO cell line expressing recombinant human muscarinic receptors *in vitro*, the number of muscarinic receptors tends to decline with time. Sapogenin derivatives of the invention (1-10 μ M) incubated for 72 hours increase muscarinic receptor density.

Methods:

- 19 -

Effect of sapogenin derivatives of the invention on muscarinic receptor density in CHO cells expressing recombinant human muscarinic receptors.

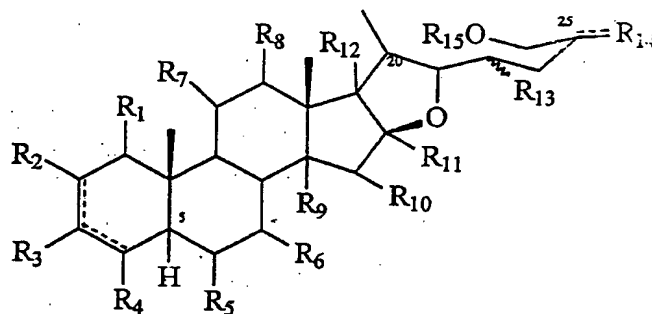
5 Chinese hamster ovary (CHO) cells expressing high levels of receptor (~2.2 pmoles receptor/mg protein) were cultured in flasks (150 ml) for 24 hours before the start of the experiment. Vehicle (DMSO) and sapogenin derivatives (at 1 and 10 μ M) were added to the medium for 48 h. The culture medium was discarded, the cells scraped off and resuspended in Hanks solution, centrifuged and m-receptor levels determined by incubating with [3 H]-QNB for 30 min followed by liquid scintillation counting.
10 Protein levels were determined by a micro Lowry method.

Results:

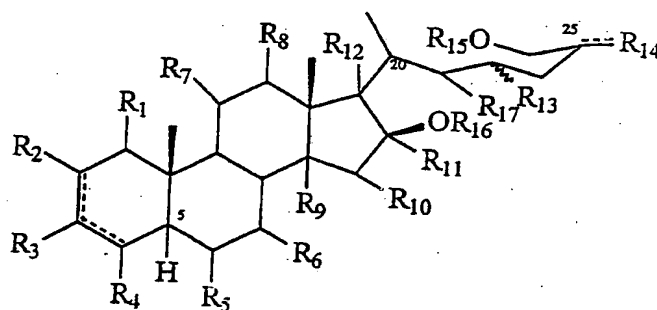
15 These are illustrated in Figure 1. Over the culturing period treatment with sapogenin derivatives of the invention prevents the decrease in muscarinic receptor number in a concentration-dependent manner.

CLAIMS:

1. Sapogenin derivatives of general formula I or II :



I



II

and their stereoisomers and racemic mixtures, their pharmaceutically acceptable pro-drugs and salts,

wherein:

in the general formula (I):

-R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, are, independently of each other, either H, OH, =O, and OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;

-R₉, R₁₂, R₁₁, R₁₃ can be either a H, OH, OR where R = alkyl or acyl group;

-R₁₄ = optionally substituted alkyl group

-R₁₅ = H, optionally substituted alkyl or optionally substituted acyl

- 21 -

-the hydrogen at C5 may be either α or β
and represents an optional double bond,

and wherein in the general formula (II):

- 5 -R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₁₀, R₁₇, are, independently of each other, either H, -OH, =O, or OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
-R₉, R₁₂, R₁₁, R₁₃ can be either a H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
10 -R₁₄ = optionally substituted alkyl group;
-R₁₅ = H, optionally substituted alkyl or optionally substituted acyl;
-R₁₆ = H, optionally substituted alkyl or optionally substituted acyl;
-the hydrogen at C5 may be either α or β
and represents an optional double bond,

15

2. The derivatives according to claim 1, wherein in the general formula (I):

- R₄, R₉, R₁₂, R₁₃ = H
-R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₁₀, can be independently of each other either H, OH, =O, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
20 -R₁₁ = H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
-R₁₄ = alkyl group
-R₁₅ = H, alkyl or acyl
25 -the hydrogen at C5 may be either α or β
and represents an optional double bond.

25

3. The derivatives according to any of the preceding claims wherein in the general formula (II):

- 30 -R₄, R₉, R₁₂, R₁₃ = H
-R₁, R₂, R₃, R₅, R₆, R₇, R₈, R₁₀, R₁₇, can be independently of each other either H, OH, =O, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
-R₁₁ = H, OH, OR where R = optionally substituted alkyl, optionally substituted acyl, optionally substituted carbamoyl, alkoxycarbonyl;
35 -R₁₄ = optionally substituted alkyl group

35

- 22 -

-R₁₅ = H, optionally substituted alkyl or optionally substituted acyl

-R₁₆ = H, optionally substituted alkyl or optionally substituted acyl

-the hydrogen at C5 may be either α or β

and represents an optional double bond.

5

4. The derivatives according to any of the preceding claims wherein in the general formula (II):

-R₁ = R₂ = R₄ = R₅ = R₆ = R₇ = R₈ = R₁₀ = R₁₁ = R₉ = R₁₂ = R₁₃ = R₁₅ = R₁₆ = R₁₇ = H,

10 -R₃ = H, OH, or OCOCH₃, or =O

-R₁₄ = CH₃.

15

5. The use of any sapogenin derivatives of formula (I) or (II) as defined in any of the preceding claims in the manufacture of a medicament for increasing the muscarinic receptor number or enhancing the function of muscarinic receptors in a human or non-human animal.

20

6. The use according to claim 5, wherein said medicament is for treating cognitive disfunction.

25

7. The use according to claim 5 or 6, wherein said medicament is for enhancing cognitive function in a patient suffering from age-related cognitive disfunction.

30

8. The use according to any of claim 5, 6 or 7, wherein said medicament is for treating a disease chosen from: Alzheimer's disease, senile dementia of the Alzheimer's type, Parkinson's disease, Lewi body dementia, postural hypotension, autism, chronic fatigue syndrome, Myasthenia Gravis, Lambert Eaton disease, diseases and problems associated with Gulf War Syndrome, occupational exposure to organophosphorus compounds and problems associated with ageing.

35

9. The use according to any of claim 5 to 8, wherein said medicament is for the treatment of a condition characterised by the presence of neurofibrillary tangles and/or β -amyloid plaques.

10. The use according to any of claim 5 to 9, wherein said medicament is for

- 23 -

treating a disease chosen from Alzheimer's disease or senile dementia of the Alzheimer's type.

5 11. A pharmaceutical composition having cognitive function enhancing properties which comprises a pharmacologically effective amount of a sapogenin derivative of formula (I) or (II) as defined in any of claims 1 to 4.

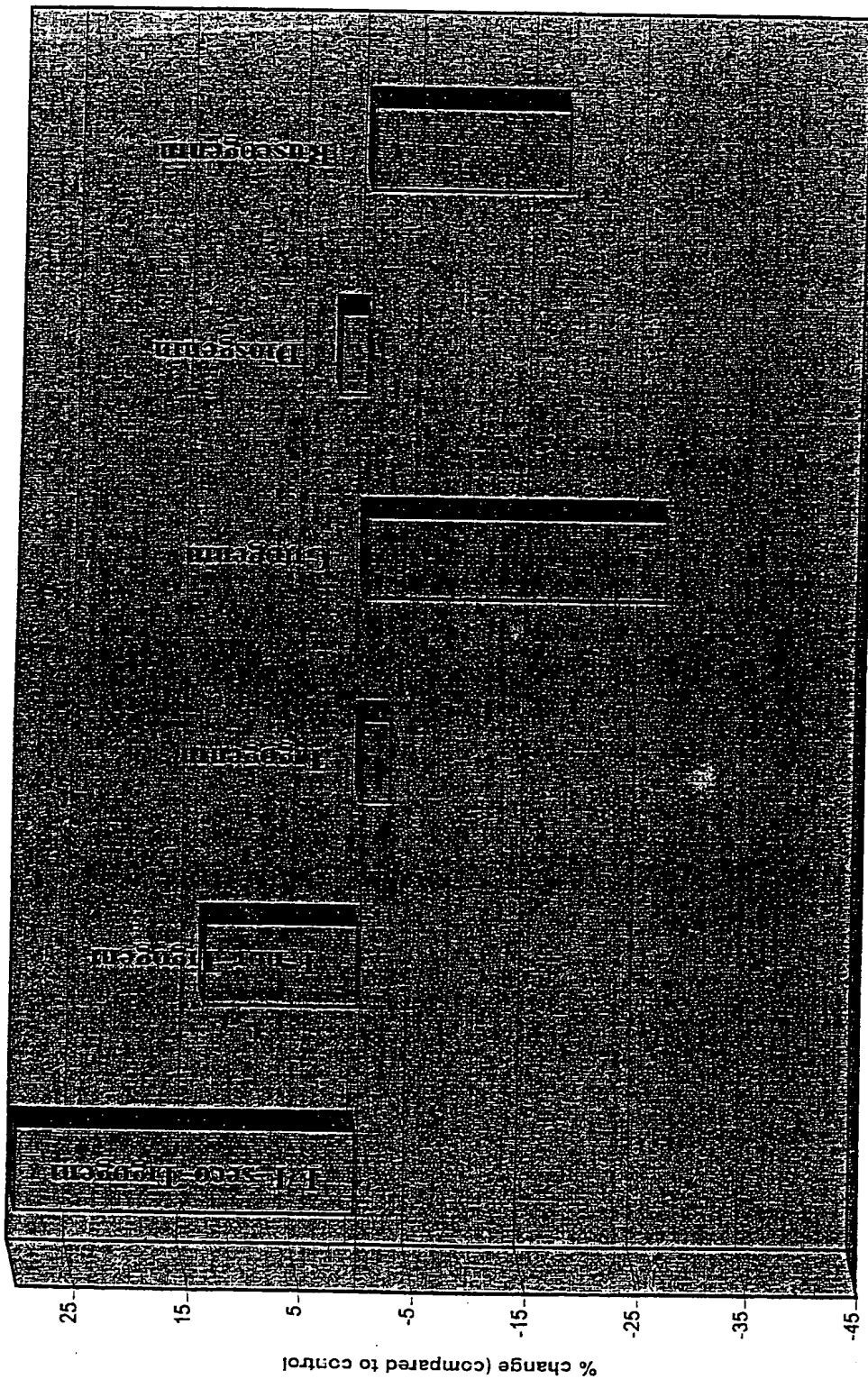
10 12. A pharmaceutical composition having cognitive function enhancing properties which comprises a pharmacologically effective amount of a sapogenin derivative of formula (I) or (II) as defined in any of claims 1 to 4 in the form of an extract derived from a plant of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave.

15 13. A non-therapeutic method of enhancing cognitive function which comprises administering to a human or animal an effective dosage of a sapogenin derivative of formula (I) or (II) as defined in any of claim 1 to 4.

20 14. The use of a sapogenin derivative of formula (I) or (II) as defined in any of claims 1 to 4 in a food product or beverage to enhance cognitive function.

1/2

Figure 1. Changes in m2 muscarinic receptor numbers following treatment at 10 μ M for 3 days



2/2

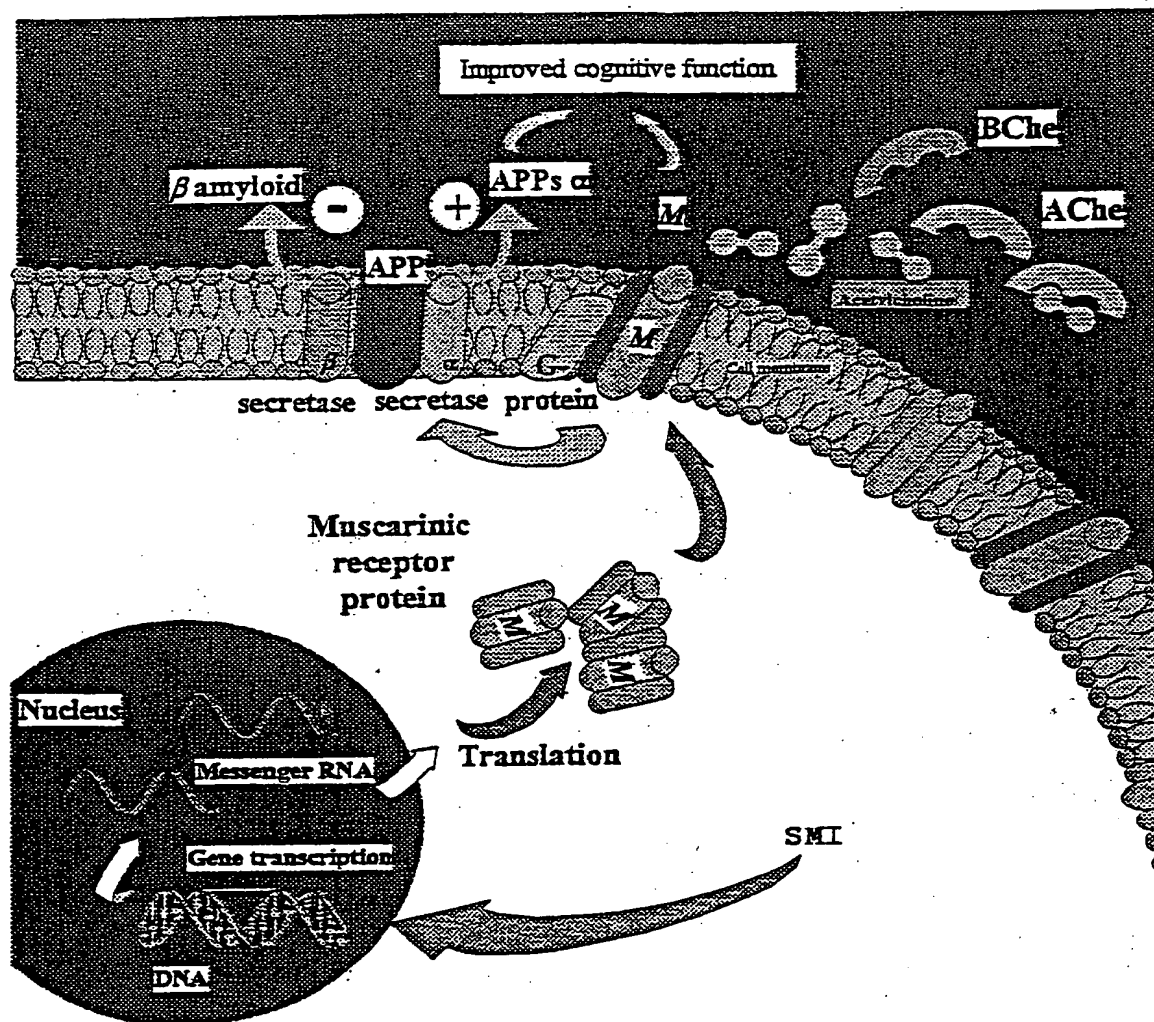


FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/03745

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07J71/00 C07J9/00 A61K31/575 A61K31/58 A61P2/04
A61P25/28 A61P25/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07J A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, BEILSTEIN Data, EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. ZACHIS ET AL: "The Clemmensen Reaction of Tigogenin. A Reinvestigation" TETRAHEDRON LETTERS, vol. 21, no. 39, 1980, pages 3735-3738, XP002156758 OXFORD GB page 3737; examples 2B,3,6 --- -/-	1-4



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

9 January 2001

Date of mailing of the international search report

06/02/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Watchorn, P

INTERNATIONAL SEARCH REPORT

Intern nal Application No

PCT/GB 00/03745

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>M. IORIZZI ET AL: "Investigation of the polar steroids from an Antarctic Starfish from the family Echinasteridae: isolation of twenty seven polyhydroxysteroids and steroidal oligoglycosides, structures and biological activities"</p> <p>TETRAHEDRON., vol. 52, no. 33, 1996, pages 10997-11012, XP002156759 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4020 page 10998; examples 16,18; table 1 page 11005; example 18</p>	1,2
X	<p>R. TSCHESCHE ET AL: "Synthese von (25S)-16.beta.,26-Dihydroxycholesterol und (25S)-16.beta.,26-Dihydroxy-5-Cholestanol"</p> <p>TETRAHEDRON LETTERS., no. 12, 1974, pages 967-970, XP002156760 ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM., NL ISSN: 0040-4039 page 967; example II page 968; example VIII</p>	1,3,4
X	<p>R. E. MARKER ET AL: "Sterols. CXLVIII. Sapogenins. LXII. The Structure of the Side Chain in the Dihydro-pseudosapogenins"</p> <p>JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 64, July 1942 (1942-07) - December 1942 (1942-12), pages 1655-1658, XP002156761 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC., US ISSN: 0002-7863 page 1656; examples I,III</p>	1,2
X	<p>A. H. ALBERT: "Reduction of the Steroidal Sapogenin Spiro Ketal System"</p> <p>JOURNAL OF ORGANIC CHEMISTRY., vol. 38, no. 12, 1973, pages 2197-2201, XP002156762 AMERICAN CHEMICAL SOCIETY. EASTON., US ISSN: 0022-3263 page 2199; examples 2A,B,C,G</p>	1,2
	<p>----- -/--</p>	

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/GB 00/03745

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. R. PETTIT: "Steroids and Related Natural Products. VII. Boron Trifluoride Etherate-Lithium Aluminium Hydride Reduction of Smilagenin Acetate" JOURNAL OF ORGANIC CHEMISTRY., vol. 26, 1961, pages 4553-4556, XP002156763 AMERICAN CHEMICAL SOCIETY. EASTON., US ISSN: 0022-3263 page 4554; examples III, VIA, B, C, D ----	1,2
X	EP 1 024 146 A (INST OF RADIATION MEDICINE ACA) 2 August 2000 (2000-08-02)	12
A	whole document, in particular, page 21, compounds III, IV; Examples 1,2; page 20, paragraph 46 ; page 33, paragraphs 69,70; ----	1-11,13, 14
A	US 3 956 491 A (ISAAC OTTO) 11 May 1976 (1976-05-11) column 5, paragraph 3 ----	1-14
A	DATABASE WPI Section Ch, Week 199548 Derwent Publications Ltd., London, GB; Class B01, AN 1995-366914 XP002156764 & CN 1 096 031 A (UNIV SHANGHAI NO 2 MEDICAL), 7 December 1994 (1994-12-07) abstract ----	1-14
X	N YI ET AL: "Sarsasapogenin: Mechanism in treating senile dementia" SYNTHESIS AND APPLICATIONS OF ISOTOPICALLY LABELLED COMPOUNDS, XX, XX, 14 September 1997 (1997-09-14), pages 315-320, XP002117140	12
A	page 315, paragraph 2 page 319, paragraph 2 ----	1-11,13, 14
X	PATENT ABSTRACTS OF JAPAN vol. 018, no. 001 (C-1148), 6 January 1994 (1994-01-06) & JP 05 246866 A (RUIBOSUTEII JAPAN:KK;OTHERS: 02), 24 September 1993 (1993-09-24) abstract ----	12
P,X	WO 99 48507 A (HU YAER ;XIA ZONGQIN (CN); BROSTOFF JONATHAN (GB); RUBIN IAN (GB);) 30 September 1999 (1999-09-30) whole document, in particular claim 7 -----	12

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 00 03745

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-4 (in part)

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claim(s) may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible. Consequently, the search on the compounds of claims 1-4 has been restricted to the single compound given in the examples (see the first structure in the table on page 14 of the description), which is in any case known from the prior art document Tetrahedron Letters Vol 21, pp 3735-8 (1980) (- see page 3737, compound 2b). The applicant has indeed clearly indicated on page 11, second full paragraph, that sapogenin derivatives of the invention "may also be commercially available", consequently by the applicant's own admission, the compounds of formula I and II as given in claims 1-4 are sufficiently well known in the art to be available commercially.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/03745

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1024146 A	02-08-2000	CN 1212966 A	07-04-1999
		AU 9336098 A	23-04-1999
		WO 9916786 A	08-04-1999
US 3956491 A	11-05-1976	DE 2416978 A	09-10-1975
		AT 338968 B	26-09-1977
		AT 266775 A	15-01-1977
		BE 827659 A	07-10-1975
		CA 1050427 A	13-03-1979
		FR 2266509 A	31-10-1975
		GB 1502873 A	08-03-1978
		NL 7504106 A	10-10-1975
CN 1096031 A	07-12-1994	NONE	
JP 05246866 A	24-09-1993	NONE	
WO 9948507 A	30-09-1999	AU 3157499 A	18-10-1999
		AU 3158199 A	18-10-1999
		WO 9948482 A	30-09-1999
		GB 2335599 A	29-09-1999
		NO 20004804 A	09-11-2000
		GB 2347676 A	13-09-2000

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.